Moderate Intensity Physical Exercise Effect on PMN and Macrophage Expression in Rattus Norvegicus Post Tooth Extraction

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Moderate Intensity Physical Exercise Effect on PMN and Macrophage Expression in Rattus Norvegicus Post Tooth Extraction

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Abstract

Rapid wound healing after tooth extraction is expected to be occurring. In order to accelerate wound healing process, oxygenation within tissue is needed. Moderate intensity physical exercise can improve the oxygenation of tissues. Oxygen (O2) is needed by Polymorphonuclear (PMN) and macrophages to phagocyte microorganisms and necrotic tissue.

The aim of this study is to determine the effect of moderate intensity physical exercise to PMN and macrophages expression after tooth extraction in wistar rat (Rattus norvegicus).

Rats were divided into 4 groups: 2 control group (K1 and K2) and 2 treatment group (P1 and P2). The treatment group was given moderate intensity physical exercise for 2 weeks (swim test). Group 1 consists of K1 and P1 group which is conducted for analyzed the number of PMN one day after tooth extraction. Group 2 consists of K2 and P2 group which is conducted for analyzed the number of macrophage 3 day after tooth extraction. The data was analyzed statistically using Independent t-test.

There were significant different between control and treatment in PMN and macrophage expression. The number of control group is lower than treatment group.

Moderate intensity physical exercises were decreased the number of PMN and macrophages after tooth extraction.

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Introduction

Tooth extraction is one of the most frequent treatment procedures performed by dentists.[1] Based on data from Report of Basic Health Research 2007, the percentage of tooth extraction or fillings are increases within age.[2] Wound healing is a response of our body to restore homeostasis in order to achieve stability of tissues or organs. The wound after extraction case, wound healing process will form of a functional epithelium on the surface of the tooth socket.[3,4] The purpose of wound healing is rapid wound closure and prevent scar formation without any complications.[5] Wound healing can be divided into four main phases, hemostasis and coagulation, inflammation, proliferation, and maturation (remodeling).[6,7]

In the early of inflammatory stage, polymorphonuclear leukocytes (PMN) migrate to the injured tissue.[8,9] PMN functions in the early stages of inflammation (24-36 hours after the injury). Neutrophils can produce anti-microbial substances and proteases. At the end of stage, neutrophil will go through apoptosis and phagocyte by macrophages.[7,10,11] In the end of inflammatory phase the number of macrophages are increase (2-3 days after tissue injury). Macrophages is important for phagocyte bacteria.[12] In addition macrophages also secrete growth factors and cytokines such as TNF-α, interleukin 1 (IL-1) and interleukin 6 (IL-6).[13,14,15]

Research conducted to investigate natural resources as wound healing promoting agents are plenty. Natural resources are scientifically proved to enhance healing process by various

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pathways, for example Propolis, can promote healing by inducing collagen formation.[16] Physical exercise is a different field of research especially regarding the process of wound healing. Physical exercise is a subcategory of physical activity that uses large skeletal muscles in a planned, structured, and repetitive for maintenance physical endurance.[17] Physical exercise can improve the health of the body depends on frequency, intensity, type, and time. The recommended frequency of training is 3-4 times a week.[18]

Moderate intensity physical exercise can improve VO_2 max resulting in better oxygenation to the tissues. Oxygenation in the injured tissue is a major determinant on wound healing.[19,20] O_2 play an important role in activation and metabolism processes of PMN and macrophages.[21] The aim of this study is to determine the effect of moderate intensity physical exercise to PMN and macrophages expression after tooth extraction in Wistar rat (Rattus norvegicus).

Materials and methods

Study Design

This study is an experimental laboratory research which conducted at the Laboratory of Biochemistry Faculty of Medicine, Universitas Airlangga. This study has been approved with ethical clearance from Committee of Ethical Clearance of Health Research, Faculty of Dental Medicine, Universitas Airlangga (No: 103/KKEPK.FKG/VII/2016). The samples were 28 male Wistar rats, 250-300 g, aged 2-3 months.

Animal Treatment

Rats were divided into 4 groups: 2 control group (K1 and K2) and 2 treatment group (P1 and P2). The treatment group was given moderate intensity physical exercise for 2 weeks (swim test). Moderate intensity physical exercise can be defined as exercising with 50% of the rat's maximum swim capacity (MSC). MSC was determined by given rats weighing 3% of its body weight and then were put in a tub of water. Time which takes the rat to show the fatigue (air bubbles while submerged/drowning) is considered the MSC.

The treatment group (P1 and P2) was given physical exercise with moderate intensity and 3% body weights while swimming with 3x swim intervals and 2x resting periods (a resting period is ½ the time of one swimming interval) for 2 weeks. After the treatment is finished, the rats were then going to have their mandibulars incisors extracted after being anesthetized. Afterwards, the empty socket of the extracted incisors was irrigated using aquadest and then sutured.

Hematoxylin Eosin (HE) Analysis

The first day after tooth extraction, control group (K1 dan P1) were anesthetized and mandible was taken for HE analysis (PMN counted). After 3 days post tooth extraction, treatment group (K2 dan P2) were anesthesized and have their mandible taken for HE analysis (macrophage counted). PMN and macrophage counted using light microscope with 400x magnification. Data obtained from the experiment was then analyzed using an Independent t-test.

Results

Expression of PMN and macrophage in each group are shown in Figure 1 and 2. PMN and macrophage counted in each group are shown in Table 1.

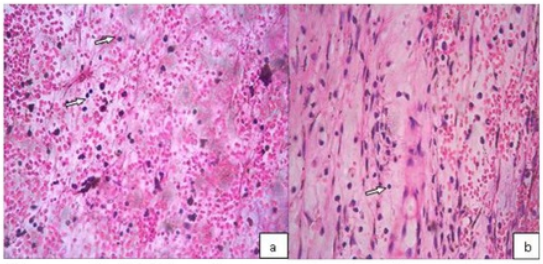

Figure 1. PMN cell on post extraction socket of Mandibular Incisive Rattus Novergicus with HE staining and 400x magnification. **(a).** Control Group (K1) **(b).** Treatment Group (P1).

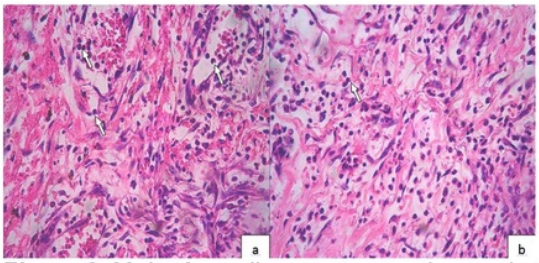

Figure 2. Makrofag cell on post extraction socket of Mandibular Incisive Rattus Novergicus with HE staining and 400x magnification (a) Control Group (K2); (b). Treatment Group (P2).

The results showed the number of PMN and macrophages in the treatment group are lower compared to control group. Independent t-test results showed that there is a significant different between P1 and K1 group on PMN counted with p=0,001(p<0,05). Independent t-test results also showed that there is a significant different between P2 and K2 group on macrophage counted with p=0,000(p<0,05).

Group	Mean	SD	n
K1	273.28	56.103	7
P1	172.42	24.985	7
K2	5.42	1.272	7
P2	1.14	0.899	7

Table 1. Mean ± SD of Group 1 for PMN and Group 2 for Macrophage.

Discussion

In this study moderate intensity physical exercise are represented by swimming due to rat ability to swim very well for a long time. Swimming is also a kind of aerobic exercise with a low-moderate intensity that can be done continuously. Physical exercise is expected to increase the supply of oxygen into the body so VO_2 max and tissue oxygenation are increased.[22,23]

The results of this study showed that moderate intensity physical exercise reduces the number of cells PMN and macrophages after tooth extraction. The previous study also showed that level of inflammation is decreased.[24] Acceleration of wound healing can occur through three mechanisms: hypoxic conditions at the beginning of the wound which stimulate the release of cytokines, decreased levels of inflammation, and increase the amount of reactive oxygen species (ROS). Increased ROS level leads to antioxidant enzymes enhancement which protect scar tissue against damage from excess ROS or free radicals. Oxygen also plays an important role to accelerate the wound healing process, especially in chronic wounds.[21,25,26]

Moderate intensity physical exercises reduce inflammatory markers through several mechanisms: reduction of pro-inflammatory cytokines (TNF-α, MCP-1, IL-6, IL-1β), lowering TLRs (Toll like receptors) that secrete pro-inflammatory cytokines, and inhibit macrophage infiltration. PMN and macrophages are inflammatory cells which is important for **phagocytosis.** Moderate intensity physical exercise may increase macrophage (M2) infiltration. M2 will secrete and increase the concentration of cytokines and growth factor (TNF-α, IL-6, TGF-β and IL-10, VEGF).[27]

Disruption of blood vessel and activation inflammatory cell in wound are will lead to hypoxic, hypoxic conditions. Hypoxic conditions in the acute injury can activate the HIF-1 (Hypoxia Inducible Factor-1), which initiates the process of gene expression that is responsive to hypoxia, such as genes VEGF, erythropoietin (EPO), TGF-β, TNF-α and PDGF which will all accelerate wound healing process.[25] Hypoxic conditions should eliminate rapidly. Wound healing process need tissue oxygenation. Tissue oxygenation can be increased by doing aerobic exercise continuously which lead to increase VO_2 max level. In addition, PMN requires a lot of oxygen to be able to function optimally.[21]

Conclusions

Moderate intensity physical exercises were decreased the number of PMN and macrophages after tooth extraction.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

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